

Gonadotropin-Releasing Hormone Secretion into Third-Ventricle Cerebrospinal Fluid of Cattle: Correspondence with the Tonic and Surge Release of Luteinizing Hormone and Its Tonic Inhibition by Suckling and Neuropeptide Y¹

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ABSTRACT

Objectives of the current studies were to characterize the pattern of GnRH secretion in the cerebrospinal fluid of the bovine third ventricle, determine its correspondence with the tonic and surge release of LH in ovariectomized cows, and examine the dynamics of GnRH pulse generator activity in response to known modulators of LH release (suckling; neuropeptide Y [NPY]). In ovariectomized cows, both tonic release patterns and estradiol-induced surges of GnRH and LH were highly correlated ($0.95; p < 0.01$). Collectively, LH pulses at the baseline began coincident with (84%) or within one sampling point after (100%) the onset of a GnRH pulse, and all estradiol-induced LH surges were accompanied by corresponding surges of GnRH. A 500- μ g dose of NPY caused immediate cessation of LH pulses and lowered ($p < 0.001$) plasma concentrations of LH for at least 4 h. This corresponded with declines ($p < 0.05$) in both GnRH pulse amplitude and frequency, but GnRH pulses were completely inhibited for only 1.5–3 h. In intact, anestrus cows, GnRH pulse frequency did not differ before and 48–54 h after weaning on Day 18 postpartum, but concentrations of GnRH ($p < 0.05$) and amplitudes of GnRH pulses (4 of 7 cows) increased in association with weaning and heightened secretion of LH. We conclude that the study of GnRH secretory dynamics in third-ventricle CSF provides a reasonable approach for examining the activity and regulation of the hypothalamic pulse generator in adult cattle. However, data generated using this approach must be interpreted in their broadest context. Although strong neurally mediated inhibitors of LH pulsatility (suckling; NPY) had robust effects on one or more GnRH secretory characteristics in CSF, only high doses of NPY briefly abolished GnRH pulses. This implies that the GnRH signal received at the hypophyseal portal vessels under these conditions may differ quantitatively or qualitatively from those in CSF, and theoretically would be undetectable or below a biologically effective threshold when LH pulses are absent.

INTRODUCTION

The ability to measure GnRH secretion in mammals provides a powerful tool for monitoring the central regulation of reproduction. Key events, including the onset of puberty, ovulation, and resumption of cyclic activity after parturition, are governed by the pattern of GnRH secretion and its electrophysiological correlates within the hypothalamus

[1]. Although a transnasal, transsphenoidal approach has been described for collecting mixed hypophyseal portal and cavernous sinus blood in ewes and young calves to monitor GnRH release [2, 3], the complex anatomical architecture of the cranium presents a significant barrier to the practical application of this method in adult cattle. Additionally, push-pull perfusion techniques have been used to obtain median eminence perfusates in the rat [4], rabbit [5], and sheep [6]; but these methods, to our knowledge, have not been reported in cattle for measuring GnRH. Consequently, the pattern of GnRH secretion in adult cattle has not been documented.

In contrast to earlier reports [7, 8], recent evidence in the monkey [9], rabbit [5], and sheep [10] demonstrated the presence of GnRH in third ventricular cerebrospinal fluid (CSF), and patterns of release corresponding directly or in delayed temporal sequences with that of LH. Moreover, the simultaneous measurement of GnRH pulsatility in third-ventricle CSF and hypophyseal portal blood of the ewe has now been reported [11]. The present study reports a technique for cannulating and sampling CSF from the third ventricle of adult cattle, and the detection and quantitation of GnRH secretion. Physiological objectives were to examine the correlation of GnRH and LH pulsatility using three experimental models: 1) ovariectomized cows, 2) ovariectomized cows implanted with estradiol and treated with the potent inhibitor of LH release neuropeptide Y (NPY), and 3) intact, anestrus females before and after the weaning-induced increase in LH.

MATERIALS AND METHODS

All animal-related procedures employed in this study were approved by the Institutional Agricultural Care and Use Committee (IAACUC) of the Texas A&M University System (Protocol No. 246), and the Guiding Principals for the Care and Use of Research Animals, of the Society for the Study of Reproduction.

Cows used in these studies were maintained in pens measuring 25 \times 9 m and were fed daily with hay and a concentrate supplement formulated to meet National Research Council recommendations for maintenance or lactation [12] and to maintain a body condition score of 5–6 (1 = emaciated; 9 = obese).

Surgical Cannulation of the Third Ventricle

This procedure was based on methods initially described for implanting electrodes in discrete regions of the hypothalamus [13] and for cannulating the ventricle of young heifers [14]. Twelve hours before surgery, prophylactic dos-

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es of sulfadimethoxine (0.14 g/kg, oral) and oxytetracycline HCl (10 mg/kg, i.v.) were administered. Animals were sedated with xylazine (20 mg, i.v.); atropine (0.67 mg/kg, i.m.) was administered to prevent excessive salivation; and the surgical site was prepared for sterile surgery. The cow was then placed in lateral recumbency and intubated, and anesthesia was induced by intravenous injection of sodium thiopental (5%) and guaifenesin (0.2%) in lactated ringers to effect. Cows were maintained under deep anesthesia by a closed circuit system of halothane (2–4%) and oxygen (4–5 L/min).

The cow was placed in sternal recumbency on a specially constructed cart, and the head was immobilized in a holder and leveled using ear bars placed in the external auditory meatus. To aid in stereotaxic positioning of the cannula based on radiographs, a 16-gauge needle was set perpendicular to the dorsal surface of the head along the midsagittal line at a position one fourth of the distance from the orbital intersect to the poll. The orbital intersect was the intersection of the midsagittal line with a line connecting the caudal limits of the right and left orbits. Using anatomical landmarks of the lateral radiograph (MinXray-300; MinXray Inc., Evanston, IL), the position of the third ventricle was estimated in relationship to the 16-gauge needle.

Using aseptic techniques, a sterile polyvinylchloride ring was centered over the calculated point of entry. Skin and muscle in this area were retracted down to the periosteum, and the sagittal suture was exposed. A 9.5-mm hole was drilled at the target site through the frontal bones and sinus cavity, and down to the dura, using a bit attached to an electrode manipulator (David Kopf Instruments, Tujunga, CA). The bit was set at the same angle as the frontal bones (usually 18–26 degrees). A stainless steel or aluminum guide cannula with an occluding stylet was lowered through the hole to the third ventricle. The stylet was removed, and the guide cannula was raised or lowered until CSF flowed vigorously from the cannula. Polyethylene tubing (0.58-mm i.d. \times 0.97-mm o.d.; Becton Dickinson, Sparks, MD) or silicone elastomer tubing (Silastic; 0.51-mm i.d. \times 0.95-mm o.d.; Königsgberg Instruments, Inc., Pasadena, CA) was threaded into the guide cannula. The guide cannula was usually raised 3–5 mm as the tubing was inserted to allow the tubing to coil within the ventral ventricle. Silicone adhesive was used to seal the tubing to the guide cannula at its exit. The location of the cannula was verified radiographically by withdrawing several milliliters of CSF and replacing them with equal volumes (1–3 ml) of radiopaque dye (Omnipaque, 240–350 mg/ml; Sanofi Winthrop, New York, NY). In a few cases the guide cannula was removed entirely, leaving only Silastic tubing coiled at the bottom of the ventricle. The polyvinyl chloride ring was placed into the circumscribed area surrounding the guide cannula, and the ring and cannula were cemented to the frontal bones with hoof repair acrylic cement (Technovit; Jorgensen Laboratories, Loveland, CO). A plug was placed in the end of the Silastic tubing, the tubing was coiled within the polyvinyl chloride ring, and a flat plastic cover was placed over the ring and retained with screws to protect the cannula. Postsurgically, rectal temperatures were recorded every morning. If rectal temperature remained at or below 39°C, the animal was administered prophylactic oxytetracycline (LA 200, 10 mg/kg i.m.) daily for 3 days after surgery. If rectal temperature rose above 39°C, or other signs of infection or inflammation (anorexia, lethargy, ataxia) were ap-

parent, a more aggressive therapeutic protocol was followed as determined by symptoms.

Experiment 1: Tonic and Surge Release of GnRH in Ovariectomized Cows

In this experiment, we tested the hypothesis that the pattern of GnRH secretion in third-ventricle CSF would be highly correlated with pulses of LH and with the preovulatory LH surge in peripheral blood. Six crossbred (Brahman \times Hereford F₁) pluriparous cows were ovariectomized at least 1 mo before cranial surgery using a standing paralumbar approach.

A 14-day recovery period was allowed between cranial surgery and the start of each experiment. Before sampling, animals were administered prophylactic doses of antibiotics as previously described and restrained in a stanchion. Using aseptic procedures, a blunt 22-gauge needle was attached to the proximal end of the cannula for CSF collection during phase 1 (tonic secretion). CSF (400–600 μ l) was collected at 10-min intervals simultaneously with jugular blood samples for 6 h. Samples were stored immediately at –70°C until assayed for GnRH. Jugular blood was collected via an indwelling jugular catheter (polyethylene tubing, 1.4-mm i.d., 0.19-mm o.d.; Becton Dickinson, Parsippany, NJ) placed at least 24 h before the start of the experiment.

Several weeks after completion of phase 1, 5 of the cows were treated i.m. with 1 mg estradiol-17 β to induce a preovulatory-like surge of LH (phase 2). Beginning 10 h after injection, blood and CSF were collected simultaneously at 30-min intervals until 18 h postinjection, and at 15-min intervals for an additional 12–18 h. Blood plasma and CSF were analyzed for GnRH and LH, respectively.

Experiment 2: Effects of Third Ventricular Infusion of NPY on GnRH and LH Secretory Dynamics in Ovariectomized, Estradiol-Implanted Cows

Three mature cows (Brahman \times Hereford, F₁) maintained in excellent body condition were ovariectomized, and an s.c. Silastic implant containing crystalline estradiol was placed in one ear of each cow. Implants were designed to produce a physiological baseline of 2–6 pg/ml of plasma [15]. Approximately 2 wk later, cows were fitted surgically with third-ventricle cannulas and assigned randomly to receive 0, 50, and 500 μ g porcine NPY (Peninsula Laboratories, Belmont, CA) in a Latin square arrangement (e.g., all cows received all treatments). During each of three treatment periods, spaced at least 2 days apart, single blood (10 ml) and CSF (600 μ l) samples were collected from each cow immediately before infusion of NPY or saline vehicle (0.3% BSA, 0.9%) into the third ventricle. Hormone and control treatments were delivered in a volume of 100 μ l. Additional blood and CSF samples were collected at 10-min intervals for 4 h, and blood plasma and CSF were analyzed for GnRH and LH, respectively.

Experiment 3: GnRH Secretion Before and After Weaning in Intact, Anestrous Cows

Third-ventricle cannulae were surgically installed in seven crossbred (five Brahman \times Hereford, F₁ and two 1/4 Brahman \times 1/4 Hereford \times 1/2 Angus) cows on Day 270 of gestation. Cows ranged in age from 3 to 12 yr of age and were either primiparous (n = 2) or pluriparous (n = 5). After surgery, cows were maintained as in experiment

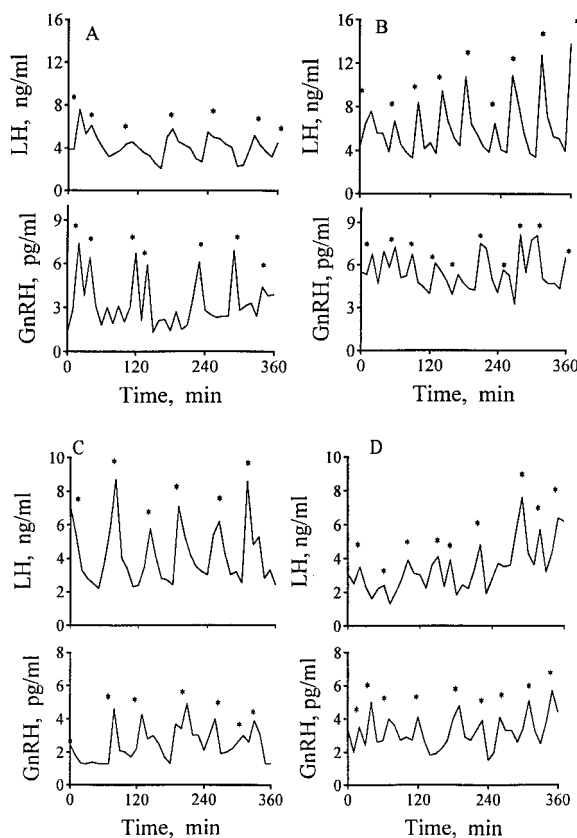


FIG. 1. Patterns of CSF GnRH (bottom panels) and LH (top panels) secretion in four representative ovariectomized cows in experiment 1 (A–D). *Synchronous pulses of both hormones as detected by a pulse detection algorithm [20].

1. On Day 18 postpartum, jugular blood and third-ventricle CSF were sampled at 10-min intervals for 6 h. Cows were weaned, and 48–54 h later (Day 21) the sampling process was repeated. All plasma and CSF samples were analyzed for LH and GnRH, respectively. Transrectal ultrasonography and serum concentrations of progesterone were used to verify that all cows were anovulatory before the study.

RIAs

GnRH was measured in duplicate 75- to 150- μ l CSF samples as described by Ellinwood et al. [16]. The iodination and other assay modifications have been described in detail elsewhere [17]. Antiserum R-1245 (Dr. Terry Nett, Colorado State University, Fort Collins, CO) was used as the source of first antibody at a final dilution of 1:16 000. The sensitivity of the assay was 0.5 pg/ml, and average intra- and interassay coefficients of variation (CV) were 3% and 15%, respectively.

Plasma concentrations of LH were determined in duplicate 200- μ l aliquots as previously described [18]. The sensitivity of the assay averaged 0.1 ng/ml, and average intra- and interassay CV were 3% and 13%, respectively. Serum progesterone concentrations were determined in single blood samples as previously described [19], except that the antiserum was GDN-337 (Dr. Gordon Niswender, Colorado State University, Fort Collins). Sensitivity was 0.05 ng/ml with intra- and interassay CV of 9% and 12%, respectively.

Pulse Detection and Data Analysis

Pulses of GnRH and LH were identified using Pulsefit 1.2, a pulse detection algorithm [20]. Temporal coinci-

TABLE 1. Pulse characteristics (mean \pm SEM) of CSF GnRH and plasma LH in ovariectomized cows.

Characteristics	CSF GnRH	Plasma LH
Baseline concentration ^a	2.0 \pm 0.4	2.1 \pm 0.5
Overall concentration ^a	2.8 \pm 0.3	3.2 \pm 0.6
Pulse amplitude ^a	2.2 \pm 0.3	1.5 \pm 0.5
Pulse frequency ^b	1.4 \pm 0.1	1.3 \pm 0.1
Interpulse interval ^c	44.7 \pm 3.4	47.2 \pm 4.9

^a Pg/ml and ng/ml for GnRH and LH, respectively.

^b Pulses/h.

^c Min.

dences between GnRH and LH pulses within cows were determined using criteria defined by Woller and coworkers [21], with the modification that the window of synchrony was expanded to within two sampling points. Temporal coincidences between GnRH and LH pulses between cows (mismatched or random data sets) were used to determine the degree of random coincidence, i.e., the synchrony between CSF GnRH and LH pulses that would occur randomly without physiological causality. A Student's *t*-test was then used to determine the significance of the difference between the random and observed synchrony [22]. For experiment 2, effects of NPY dose, time, and the dose \times time interaction on hormone concentrations, pulse frequencies, and amplitudes were assessed by ANOVA as for a Latin square design [22] using the PROC GLM procedure of the Statistical Analysis System (SAS; [23]). In experiment 3, period means were contrasted with the Student-Newman-Keuls test [22]. Mean basal concentrations, overall mean concentrations, and pulse amplitudes of GnRH and LH pre- and postweaning were compared by ANOVA for repeated measures using the general linear models procedures of SAS [23]. Pre- and postweaning period means for pulse frequency data were contrasted by using Student's *t*-tests [22].

RESULTS

Cannulation of the Third Ventricle

The distance from the caudal limit of the orbit to the poll averaged (\pm SEM) 174 \pm 3.4 mm with a range of 165–185 mm in both experiments. The third ventricle was located at an average distance of 68 \pm 2.0 mm from the caudal limit of the orbit and at a depth of 82.6 \pm 2.1 mm from the frontal bone. Cannulas remained functional for up to 8 mo in some animals.

Experiment 1: Tonic and Surge Release of GnRH in Ovariectomized Cows

Tonic patterns of CSF GnRH and plasma LH secretion in four representative ovariectomized cows are shown in Figure 1. GnRH was secreted into the CSF of the third ventricle in a pulsatile pattern. Mean concentrations and pulse frequency characteristics are summarized in Table 1 for all cows. A similar pattern of CSF GnRH and plasma LH secretion was observed. Over sampling periods totaling 36 h, 44 LH pulses were identified, and 37 (84%) occurred in exact temporal synchrony or within one sample after a GnRH pulse. All LH peaks (100%) occurred within 2 sampling points after onset of a GnRH pulse. This degree of synchrony differed ($p < 0.025$) from that observed using mismatched data (mismatched data: 21% coincident; 51% within 2 samples). Interpulse intervals were similar for both hormones (Table 1).

TABLE 2. Characteristics (mean \pm SEM) of estradiol-induced surges of plasma LH and CSF GnRH in 4 long-term ovariectomized cows.

Hormone	Characteristics			
	Baseline concentration ^a	Onset of surge after injection (h)	Duration of surge (h)	Peak concentration ^a
LH	3.06 \pm 0.1	18.6 \pm 0.7	7.8 \pm 2.7	55.4 \pm 18.5
GnRH	4.7 \pm 1.4	18.6 \pm 0.7	N/A ^b	14.5 \pm 2.0

^a LH (ng/ml) and GnRH (pg/ml), respectively, before estradiol injection.^b Sampling duration inadequate to characterize.

Estradiol-induced surges of LH ranging from 14.7 to 97 ng/ml were observed in 4 of 5 cows between 18 and 21.25 h after estradiol injection. The duration of the surges varied considerably in these long-term ovariectomized females, ranging from 5 to 13.25 h (Table 2). Surges of GnRH occurred coincident with those of LH, and their magnitudes were proportional to those of corresponding LH surges. Figure 2 presents GnRH and LH secretion patterns in 2 cows exhibiting long-duration, high-amplitude LH surges and shorter-duration, less robust LH surges, respectively. Duration of sampling was adequate for characterizing the entire LH surge in all cases, but GnRH surges extending beyond those for LH were not fully characterized by our sampling regimen.

Experiment 2. Effects of Third Ventricular Injection of NPY on GnRH and LH Secretory Dynamics in Ovariectomized, Estradiol-Implanted Cows

Responses of ovariectomized, estradiol-implanted cows to third-ventricle infusions of NPY are summarized in Table 3. Saline vehicle infusions had no effect on any of the LH or GnRH variables studied. Infusion of 50 μ g NPY tended to cause a decrease ($p < 0.10$) in mean LH concentrations compared to the control. This decrease was associated with a reduction in the amplitudes of LH pulses ($p < 0.05$) but had no effect on the frequency of detectable pulses. None of the GnRH variables were statistically affected by the 50- μ g dose. At the higher NPY dose (500 μ g), an immediate cessation of LH pulsatility was observed in all cows (Fig. 3) and this was accompanied by lower mean concentrations of LH ($p < 0.001$). Changes in LH secretion at the 500- μ g dose corresponded with a decline in both the frequency and amplitude of GnRH pulses in CSF. As demonstrated in Figure 3, GnRH pulsatility ceased entirely for 1.5–3 h. After this period, a degree of GnRH pulsatility resumed although LH pulses remained completely inhibited.

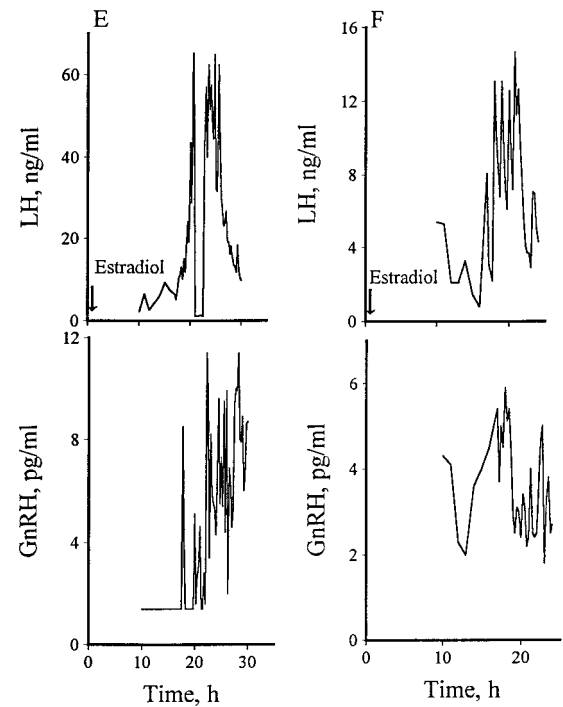


FIG. 2. Estradiol-induced GnRH (bottom panels) and LH (top panels) surges in a cow (E) exhibiting a long-duration, high-amplitude LH surge, and a cow (F) with a less robust surge. Note proportional differences in the corresponding magnitudes of GnRH surges and the different scales.

Experiment 3: GnRH Secretion Before and After Weaning in Intact, Anestrous Cows

Data summarizing mean concentrations and pulse characteristics for CSF GnRH and plasma LH in 7 cows before and after weaning are shown in Table 4. Compared to the preweaning control period, mean baseline concentrations of GnRH ($p < 0.05$), and overall mean concentrations of GnRH, ($p < 0.01$) increased postweaning, but GnRH pulse frequency did not change ($p > 0.05$). Postweaning frequencies of LH pulses and mean concentrations of LH increased ($p < 0.05$ and $p < 0.01$, respectively) relative to preweaning values, but baseline concentrations were not affected. The ascending baseline observed postweaning for GnRH in 6 of 7 cows could be attributed to a within-animal increase ($p < 0.05$) in the amplitude of GnRH pulses in 4 of 7 cows. Figure 4 shows the pattern of GnRH secretion observed pre- and postweaning, coincident with increased LH pulse frequency, in two representative cows. In all

TABLE 3. Effects of third ventricular infusion of NPY on tonic secretion characteristics (mean \pm SEM) of plasma LH and CSF GnRH in 3 ovariectomized, estradiol-implanted cows treated with 0 (saline), 50, and 500 μ g NPY in a Latin square arrangement.

Dose of NPY (μ g)	Hormone characteristics					
	LH			GnRH		
	Concentration (ng/ml)	Amplitude (ng/ml)	Frequency (pulses/h)	Concentration (pg/ml)	Amplitude (pg/ml)	Frequency (pulses/h)
0 (saline)	4.7 \pm 1.4	3.2 \pm 0.5	0.9 \pm 0.2	4.4 \pm 0.4	3.0 \pm 0.3	1.5 \pm 0.2
50	3.3 \pm 0.6*	1.2 \pm 0.2**	1.0 \pm 0.2	4.7 \pm 0.4	2.6 \pm 0.8	1.4 \pm 0.3
500	2.8 \pm 0.5**	0***	0***	5.5 \pm 0.3	2.0 \pm 0.6**	0.9 \pm 0.3**

* Differs from control ($p < 0.10$).** Differs from control ($p < 0.05$).*** Differs from control ($p < 0.001$).

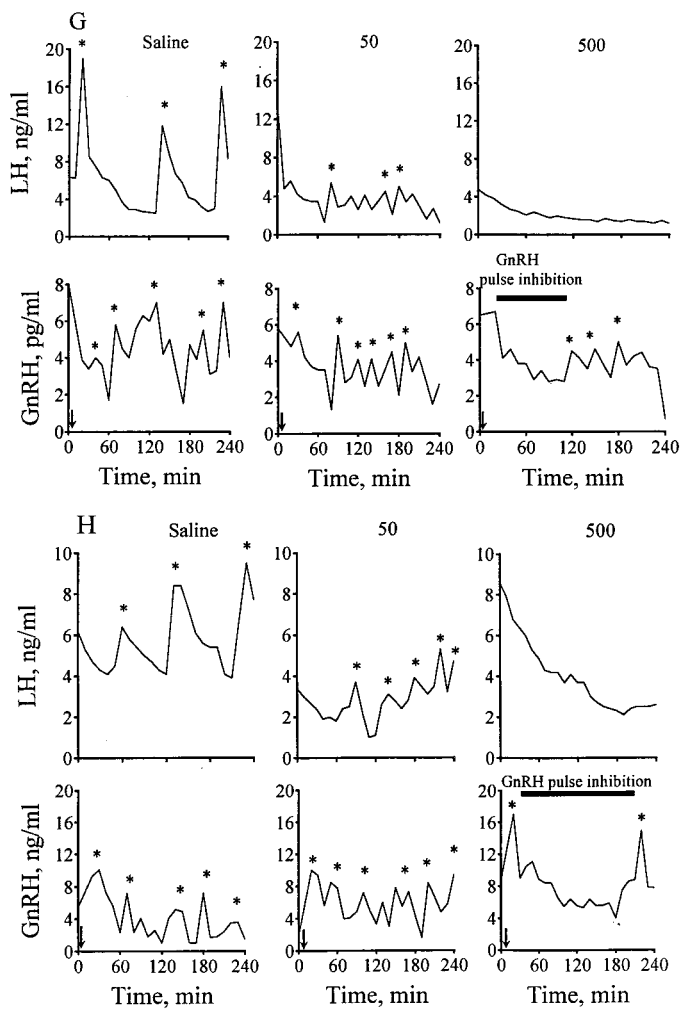


FIG. 3. Patterns of CSF GnRH (bottom panels) and plasma LH (top panels) in 2 cows (G and H) after third ventricular injection of 0, 50, and 500 μ g NPY. Note 1) the complete absence of LH pulses after the 500- μ g dose coincident with the cessation of GnRH pulses (denoted by solid horizontal bars) for 1.5 (G) to 3 (H) h, and 2) the rebound in CSF GnRH pulsatility coincident with a continued suppression of LH. See Discussion for details. *LH and GnRH pulses. Arrows, injection of NPY.

cows, the frequency of GnRH pulses was essentially identical before and after weaning, averaging 1.1–1.3 pulses/h.

DISCUSSION

Tonic Secretion in Ovariectomized Cows

Results from experiment 1 (ovariectomized cows) indicated that pulsatile secretion of GnRH in cattle can be detected by frequent sampling of CSF from the third ventricle. Recent studies have reported the detection of GnRH pulses

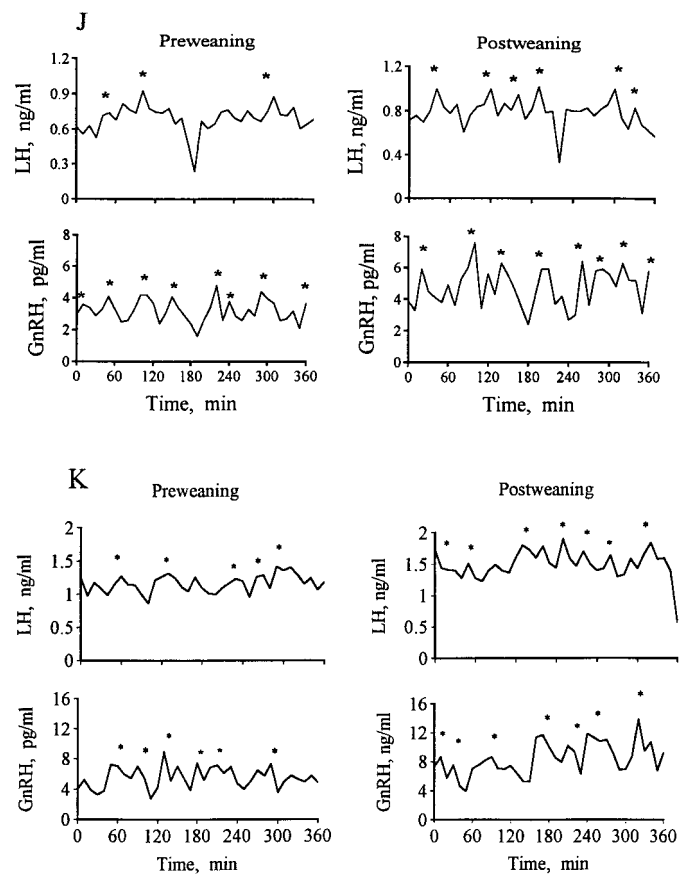


FIG. 4. Patterns of CSF GnRH (bottom panels) and plasma LH (top panels) secretion in two representative cows (J and K) before and after weaning. Blood and CSF samples were collected at 10-min intervals for 6 h on Day 18 postpartum, and after 48–54 h of weaning on Day 21. Postweaning increases in GnRH baseline and pulse amplitudes, coincident with increased LH pulse frequencies, are evident. Note that GnRH pulse frequency was ≥ 1.0 pulses/h in both cows both before and after weaning. *GnRH and LH pulses.

in the CSF of rabbits, monkeys, sheep, and rats [5, 9, 10, 24], but to our knowledge this is the first report of this phenomenon in cattle. The relatively tight temporal association between CSF GnRH and plasma LH in ovariectomized females described herein, together with remarkably similar interpulse intervals, supports a recent report in the ewe. In that study, the frequency of CSF GnRH pulses corresponded closely with both peripheral LH pulses and GnRH pulses measured in pituitary portal blood [11].

The CSF withdrawal rate (400–600 μ l/10 min) in our study did not appear to attenuate pituitary function, which agrees with observations in the ewe [10], in which the sampling rate reached 100 μ l/min. Furthermore, we have withdrawn CSF from cows for up to 20 h without any apparent

TABLE 4. Changes in CSF GnRH and plasma LH in intact, anestrus beef cows (n = 7) before and after weaning for 48–54 h on Day 18 postpartum.^a

Characteristic	CSF GnRH		Plasma LH	
	Prewaning	Postweaning	Prewaning	Postweaning
Baseline concentration ^b	2.7 \pm 0.9	4.1 \pm 0.9*	0.6 \pm 0.20	0.6 \pm 0.20
Overall concentration ^b	5.9 \pm 0.2	6.7 \pm 0.2**	0.8 \pm 0.03	1.1 \pm 0.06**
Pulse amplitude ^b	2.2 \pm 0.3	2.1 \pm 0.2	0.4 \pm 0.10	0.6 \pm 0.30
Pulse frequency ^c	1.4 \pm 0.1	1.5 \pm 0.1	0.7 \pm 0.06	1.2 \pm 0.12*

^a Comparisons are postweaning vs. preweaning: * $p < 0.05$; ** $p < 0.01$.

^b Pg/ml and ng/ml for GnRH and LH, respectively.

^c Pulses/h.

deleterious effects. In contrast, CSF sampling at rates greater than 30 $\mu\text{l}/15\text{ min}$ in the rhesus monkey, whose CSF volume is much smaller than in cattle, markedly attenuated LH secretion [9]. Reported secretion rates of CSF in other animals range from approximately 160 μl in the goat to 350 $\mu\text{l}/\text{min}$ in the human [25, 26], which probably explains why no attenuation of LH release due to the CSF sampling technique has been encountered in sheep and cattle.

The mean amplitude of GnRH pulses in experiment 1 of this study and in ewes [11] was lower than previously measured in portal blood of bull calves [3] and ewes [11], which is probably the result of the dilutional effects of CSF. CSF GnRH could represent neurohormone that diffuses across the ependymal lining of the ventricle, possibly from the median eminence, an abundant source of GnRH in cattle [27, 28]. Alternatively, GnRH may be secreted directly into the CSF; however, in the ewe, less than one percent of GnRH neurons terminate in the walls of the third ventricle [29].

The Estradiol-Induced Surge

The general pattern of GnRH release during the estradiol-induced LH surge was similar to that reported previously for the ewe in either CSF [10] or both CSF and portal blood [11]. The sampling frequency of the current study was greater than in the sheep studies (15 min vs. 30 min); therefore, the acute pattern of GnRH pulsatility during the surge was more evident (Fig. 2). The mean durations of LH and GnRH surges in this study were shorter than the mean duration of natural or estradiol-induced LH surges reported previously in cattle [30, 31]. This can probably be explained by the fact that these were long-term ovariectomized females that had not been exposed to estrogen for some time and, therefore, may not have had an adequate number of estradiol receptors to yield surges of completely normal duration. Maximum peak concentrations of LH were similar to those reported previously [30–33]. However, the mean peak concentration of GnRH in CSF was lower than that reported in the ewe [10, 11]. Cows with the lowest-amplitude, shortest-duration surges of LH (14–15 ng/ml) exhibited GnRH increases that were equally less robust, whereas larger surges of LH corresponded with proportionately larger increases in GnRH. Although sampling in the current study was adequate to describe the entire LH surge for each animal, it was not adequate to establish whether the duration of the GnRH surge consistently exceeded that for LH as reported previously in the ewe [10, 11]. However, in at least two of the animals in which the complete LH surge was captured before the termination of sampling, a trend for the GnRH surge to continue was apparent.

Central Effects of NPY

Evidence suggests that the peptide NPY acts as a neuromodulatory link between nutritional status and the central reproductive axis [34]. This peptide is a potent orexigenic agent; is highly expressed in hypothalamus, anterior pituitary, and adipose tissue; increases dramatically in CSF during undernutrition; and negatively modulates the secretion of LH when centrally infused [20, 35–37]. Effects of third ventricular infusion of NPY on LH secretion in this study were similar to those previously observed in the monkey [21], ewe [35], and rodent [38]. Although the complete suppression of LH pulses at the highest dose occurred coincident with a corresponding inhibition of GnRH pulses for up to 3 h, the continued suppression of LH was not depen-

dent upon the complete inhibition of CSF GnRH pulses. At least three possibilities could account for these effects of NPY: 1) consistent, but low-amplitude pulses of GnRH in the third ventricle after treatment are reflected as a complete absence of GnRH pulses in the hypophyseal portal system, 2) the amplitudes of corresponding GnRH pulses in portal blood are similar to those in CSF but below a threshold required to elicit LH release from the pituitary, or 3) NPY suppression of LH during periods of continued GnRH pulsatility reflects direct effects of NPY on the pituitary. Since there are NPY receptors located in the pituitary [35, 39], it is likely that high concentrations of NPY infused into the third ventricle could readily traverse the infundibular recess to enter tissues of the pituitary stalk and be transported to the pituitary gland.

Puerperal GnRH Secretion in Anestrous Cows Before and After Weaning

Suckling inhibits the pulsatile release of LH and delays first postpartum ovulation in cattle for an average of 45–60 days. Removal of calves as early as 2 wk postpartum results in a rapid rise of LH pulse frequency within 2–6 days, with the majority of cows exhibiting this response within 48 h [40, 41]. This is a very well-documented physiological phenomenon, and the third-ventricle model affords the opportunity to examine existing dogma, inferred mainly from indirect studies involving the measurement of LH, that GnRH secretion rises in concert with that of LH. The current work indicates that suckling does not strongly inhibit the rate of GnRH neuronal activity in well-nourished cattle beyond the second to third week postpartum. Although weaning-induced increases in LH pulse frequency and plasma concentrations of LH occurred coincident with increases in basal and overall concentrations of CSF GnRH and GnRH pulse amplitude, no changes in GnRH pulse frequency were observed. Whether the increase in signal strength in CSF is reflected in hypophyseal portal blood as an increased amplitude or frequency of discrete pulses will require further investigation. However, on the basis of these results, GnRH priming of gonadotrophs and, therefore, increased sensitivity to physiological levels of GnRH may be an important factor in the evolution of LH pulsatility during postpartum recovery. In the present work, a high-frequency pattern of low-level GnRH pulses in CSF occurred well before the development of a similarly frequent pattern for LH. This suggests that recovery from the effects of parturition and suckling occurs in a step-wise manner, including the repletion of anterior pituitary stores of LH [41–43] and increased GnRH pulse generator activity [44] or signal strength (current work). We believe that enhanced gonadotroph sensitivity leading to a greater frequency of LH discharge follows these events.

Summary and Overall Conclusions

In summary, the technique of third-ventricle cannulation provides a more direct assessment of the activity of the hypothalamic GnRH pulse generator of adult cattle than deduced previously by monitoring only the dynamic changes in plasma LH. Taken together, these data indicate that the third-ventricle cannulation technique will be valuable for detecting physiological changes in overall pulse generator activity. On the basis of the current studies, it is clear that high-frequency, high-amplitude pulses of LH are accompanied by similar patterns of GnRH release in CSF. Yet strong inhibitors of LH pulsatility, putatively acting at

the level of the central nervous system (suckling) or at both central nervous system and pituitary (NPY), produced periods of discordance between GnRH and LH pulses. In general, only the most potent inhibition of LH pulsatility (500 µg NPY) resulted in a total suppression of GnRH pulses in CSF, and this suppression was relatively short-lived. Under all other conditions, only the baseline and amplitudes of GnRH pulses were affected. Further studies will be required to determine how the presence of low-amplitude, high-frequency GnRH pulses in CSF, occurring in the midst of neurally mediated inhibition of LH release, are translated at the level of the hypophyseal portal system in cattle. The obvious, but unconfirmed, assumption is that corresponding signals at that site are absent or below a biologically effective threshold.

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